

Brief report

Context-dependent function of “GATA switch” sites in vivo

Jonathan W. Snow,¹⁻³ Jennifer J. Trowbridge,¹⁻⁴ Kirby D. Johnson,⁵ Tohru Fujiwara,⁵ Nikla E. Emambokus,^{1,3} Jeffrey A. Grass,⁵ Stuart H. Orkin,^{1-4,6} and Emery H. Bresnick²

¹Division of Hematology-Oncology, Children’s Hospital Boston, Boston, MA; ²Dana-Farber Cancer Institute, Boston, MA; ³Harvard Medical School, Boston, MA; ⁴Harvard Stem Cell Institute, Boston, MA; ⁵Department of Cell and Regenerative Biology, Paul Carbone Comprehensive Cancer Center, Wisconsin Institutes for Medical Research, University of Wisconsin School of Medicine and Public Health, Madison, WI; and ⁶Howard Hughes Medical Institute, Boston, MA

Master transcriptional regulators of development often function through dispersed *cis* elements at endogenous target genes. While *cis*-elements are routinely studied in transfection and transgenic reporter assays, it is challenging to ascertain how they function in vivo. To address this problem in the context of the locus encoding the critical hematopoietic transcrip-

tion factor *Gata2*, we engineered mice lacking a cluster of GATA motifs 2.8 kb upstream of the *Gata2* transcriptional start site. We demonstrate that the –2.8 kb site confers maximal *Gata2* expression in hematopoietic stem cells and specific hematopoietic progenitors. By contrast to our previous demonstration that a palindromic GATA motif at the neighboring

–1.8 kb site maintains *Gata2* repression in terminally differentiating erythroid cells, the –2.8 kb site was not required to initiate or maintain repression. These analyses reveal qualitatively distinct functions of 2 GATA motif-containing regions in vivo. (*Blood*. 2011;117(18):4769-4772)

Introduction

GATA factors are often expressed in overlapping and reciprocal patterns in hematopoiesis. Accrued evidence supports a model in which GATA factors cross-regulate transcription of their respective genes through a “GATA switch.”^{1,2} This hypothesis has been investigated at the *Gata2* locus, where several conserved GATA motif-containing regions span ~100 kb. GATA-2 has a critical role in the emergence and maintenance of hematopoietic stem/progenitor cells^{3,4,5-10} and appears to positively regulate its own transcription. During erythroid cell differentiation, GATA-1 mediates repression of *Gata2* expression. A *cis*-element located –2.8 kb upstream from the 1S *Gata2* promoter has been proposed to mediate GATA-2-dependent positive feedback before GATA-1-instigated repression.¹¹⁻¹³ Removal of this site would thus be predicted to abrogate positive feedback, reducing *Gata2* expression in hematopoietic stem/progenitor cells. We previously demonstrated that a distinct *cis*-element –1.8 kb upstream of the *Gata2* promoter maintains repression of *Gata2* in late-stage erythroblasts, though it is not required to initiate repression.¹⁴ As the patterns of GATA-factor binding at the –1.8 kb and –2.8 kb sites are similar,¹¹⁻¹³ we addressed whether the –2.8 kb site is also required for GATA-1-mediated *Gata2* repression.

We demonstrate that the –2.8 kb site is not required for initiation or maintenance of *Gata2* repression during erythroid differentiation. By contrast, the –2.8 kb site confers maximal *Gata2* expression in HSCs and progenitors in specific contexts. Comparison of the –2.8 kb and –1.8 kb sites illustrates how GATA motifs function uniquely and collectively to achieve complex and dynamic transcriptional control at the *Gata2* locus during hematopoietic development.

Methods

Generation of *Gata2* Δ-2.8 knockin mice

We replaced a 400 bp region containing 6 GATA motifs with a LoxP-PGKneo-LoxP cassette (supplemental Figure 1A, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Details of the cloning strategy are available on request. Gene targeted ES cell clones were identified by Southern blotting (supplemental Figure 1B-C). Confirmation of Cre-mediated *neo* excision was performed with PCR before blastocyst injection (supplemental Figure 1D). All mouse studies were conducted under guidelines associated with protocols approved by Harvard Medical School.

Gene expression and chromatin occupancy

Sampling of fetal liver and bone marrow cells, flow cytometric analysis and cell sorting, quantitative ChIP assay, and real-time reverse-transcriptase PCR were performed as published.¹⁴

Results and discussion

Previous studies demonstrated a critical role for *Gata2* in the emergence and maintenance of hematopoietic stem cells (HSCs).^{3,4} The –2.8 kb region drives exogenous expression of a reporter gene in HSCs in a manner dependent on GATA¹⁵ and E-box¹⁶ motifs, and has been proposed to be the primary *cis*-element conferring *Gata2* expression in these contexts.

Mice homozygous for deletion of a 400 bp region located –2.8 kb upstream of the *Gata2* 1S promoter (supplemental Figure 1) were born at expected Mendelian ratios (data not shown).

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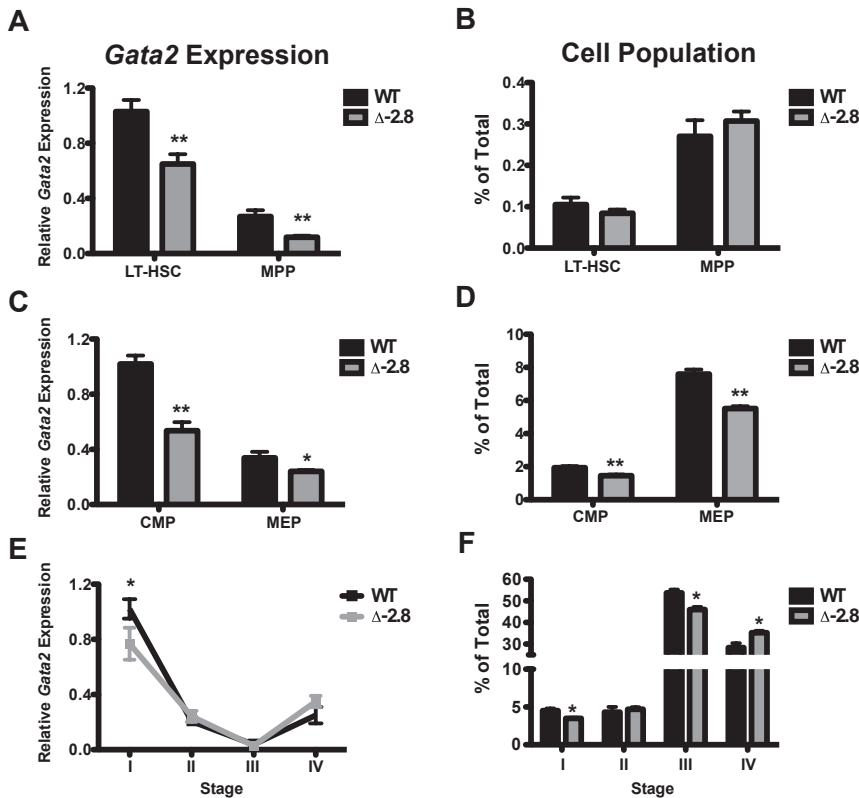


Figure 1.—2.8 kb GATA switch site regulates *Gata2* expression in hematopoietic stem and progenitor cells, but is dispensable in erythroid cells. *Gata2* expression within LT-HSC and MPP populations (LT-HSC defined as Lin^{dim}ckit⁺Sca1⁺CD150⁺ and MPP defined as Lin^{dim}ckit⁺Sca1⁺CD34⁺ or Lin^{dim}ckit⁺Sca1⁺CD150⁻) from E12.5 fetal livers normalized to β -actin (A). Frequencies of LT-HSCs and MPPs in wild-type and Δ -2.8 fetal livers (B). *Gata2* expression within CMP and MEP populations (with CMP defined as Lin^{dim}Sca1⁻,c-kit⁺CD34⁺, Fc γ R⁻ and MEP defined as Lin^{dim}Sca1⁻c-kit⁺CD34⁻, Fc γ R⁻) from E12.5 fetal livers normalized to β -actin (C). Frequencies of CMPs and MEPs from wild-type and Δ -2.8 fetal livers (D). *Gata2* expression was assessed by qPCR in Stage I through Stage IV sorted erythroid cells, corresponding to CD71^{lo}Ter119⁻ (committed erythroid progenitors, Stage I), CD71^{hi}Ter119⁻ (proerythroblasts, Stage II), CD71^{hi}Ter119⁺ (basophilic erythroblasts, Stage III), and CD71^{lo}Ter119⁺ (late erythroblasts, Stage IV) from wild-type and Δ -2.8 embryos. (E). Relative number of cells in Stage I-IV was determined in wild-type and Δ -2.8 E13.5 fetal liver cells, based on CD71 and Ter119 expression (F). Mean \pm SEM. Statistical significance was assessed by 2-sided Student *t* test. **P* \leq .05 and ***P* \leq .01.

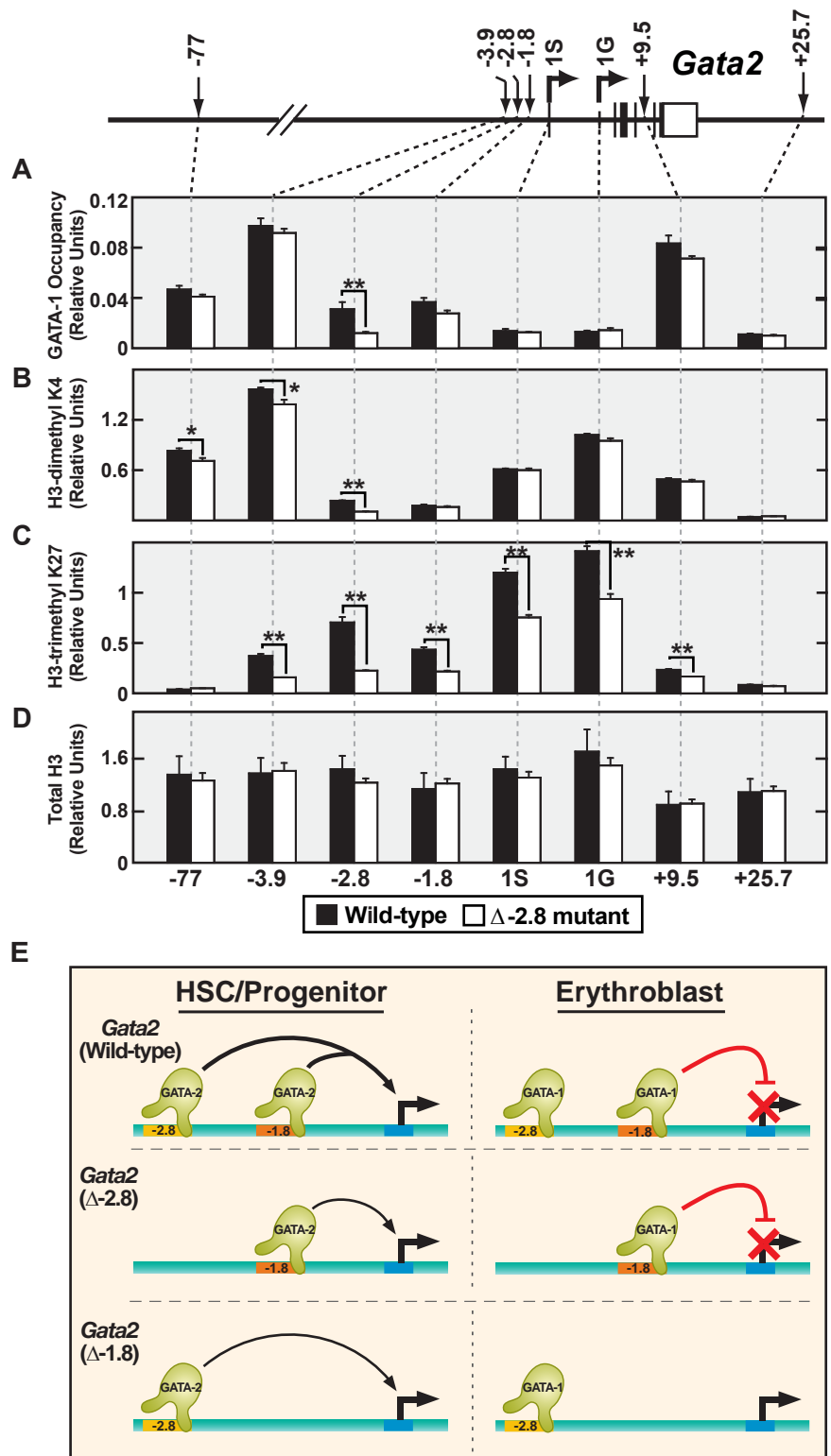
We quantified *Gata2* expression in sorted long-term HSCs (LT-HSCs) and multipotent progenitors (MPPs) from embryonic day (E) 12.5 fetal livers. Δ -2.8 LT-HSCs and MPPs exhibited reduced *Gata2* expression versus control cells (Figure 1A), but this reduction did not alter the frequency of the populations (Figure 1B). *Gata2* serves critical roles in the maintenance and specification of multipotential progenitors.⁵⁻¹⁰ Expression of *Gata2* was reduced in common myeloid progenitors (CMPs) and megakaryocyte-erythroid progenitors (MEPs) of Δ -2.8 fetal livers (Figure 1C), and was associated with a modest reduction in the frequency of these populations (Figure 1D). Quantitative ChIP analysis with enriched progenitor cells from Δ -2.8 and wild-type E14.5 fetal livers revealed a selective reduction of GATA-2 occupancy at the -2.78 kb site (surrogate for measuring occupancy at the deleted -2.8 kb site; supplemental Figure 2). These results indicate that the -2.8 kb site contributes to *Gata2* expression in fetal liver-derived multipotent and committed erythroid progenitors in part through GATA-2 binding.

To examine the function of this site at a different developmental stage, we isolated LT-HSC and MPP populations from adult Δ -2.8 and wild-type bone marrow. *Gata2* expression was reduced in adult MPPs, but not LT-HSC (supplemental Figure 3A). We observed a similar reduction in *Gata2* in MPP from Δ -1.8 mice (supplemental Figure 3A), implying some functional overlap. Loss of either site did not alter LT-HSC, MPP, CMP, GMP, or MEP numbers in adult mice (supplemental Figure 3B-C). No differences in hematopoietic colony formation or peripheral blood parameters were apparent in the Δ -2.8 or -1.8 adult mice (supplemental Table 1 and supplemental Figure 3D). Competitive primary and secondary transplant experiments revealed no statistically significant differences in the ability of Δ -2.8 or Δ -1.8 HSCs to contribute to hematopoiesis in these assays (supplemental Figure 3). Thus, while both sites confer maximal *Gata2* expression in certain contexts, they are not essential for hematopoiesis in the mouse.

Our prior work demonstrated that the -1.8 kb site maintains GATA-1-mediated repression of *Gata2* during erythroid differentiation.¹⁴ As the 2 sites have similar patterns of GATA-factor binding,¹¹⁻¹³ we investigated *Gata2* repression during erythroid differentiation in Δ -2.8 mice. *Gata2* expression was reduced in Stage I of erythroid differentiation in the Δ -2.8 mice (Figure 1E). Stage I contains MEP activity,¹⁷ consistent with the findings of Figure 1C. Despite this reduction, *Gata2* expression was normal in Stages II-IV in the Δ -2.8 fetal livers, indicating that the -2.8 kb site is not required for initiation or maintenance of GATA-1-mediated repression (Figure 1E). Only modest differences in the relative abundance of cells from each stage were apparent in Δ -2.8 versus wild-type mice (Figure 1F).

Based on the unexpected observation that the -1.8 kb, but not the -2.8 kb, site mediates repression, we reasoned that comparing their capacities to regulate nucleoprotein architecture would yield mechanistic insights. Quantitative ChIP analysis with Δ -2.8 and wild-type E14.5 fetal liver cells revealed low GATA-1 occupancy at the -2.78 kb site (Figure 2A). ChIP analysis of Pol II occupancy demonstrated no change on loss of the -2.8 kb site (data not shown), contrasting with our demonstration that loss of the -1.8 kb site results in Pol II accumulation.¹⁴ We quantified dimeH3K4 and trimeH3K27 marks associated with *Gata2* repression.^{11,13,18} Results from the Δ -1.8 mice demonstrated locus-wide reduction in dimethylH3K4 with no significant reductions in trimethylH3K27 (supplemental Figure 5A-B and Snow et al¹⁴). By contrast, loss of GATA-1 occupancy on deletion of the -2.8 kb site results in locus-wide reduction in one of the marks associated with repression, trimeH3K27 (Figure 2C), while having minimal effects on dimeH3K4 (Figure 2B), and no influence on repression. Although these data implicate a function of the polycomb repressive complex 2 (PRC2)¹⁸ at the -2.8 kb site, reduction of

Figure 2. The -2.8 kb GATA switch site establishes trimeH3K27 marks at the *Gata2* locus. Quantitative ChIP analysis of the *Gata2* locus in E14.5 fetal liver cells using antibodies to GATA-1 (A), dimeH3K4 (B), trimeH3K27 (C), and Total H3 (D). Updated model of the molecular regulation of *Gata2* repression (E). Mean \pm SEM. Statistical significance was assessed by 2-sided Student *t* test and * $P \leq .05$ and ** $P \leq .01$.



H3K27 trimethylation does not reactivate *Gata2* transcription (Figure 1).

In summary, our analyses of GATA motif function in vivo establish fundamentally different requirements for the -2.8 and -1.8 kb GATA switch elements. As GATA motifs within these sites conferred unique chromatin states and exerted different activities, these results support a model in which initiation/maintenance of *Gata2* transcriptional control requires the collec-

tive actions of dispersed GATA factor complexes with qualitatively distinct contributions (Figure 2E). As GATA-1 occupies different permutations of GATA motifs, including canonical, palindromic, composite, and noncanonical,¹⁹ our results suggest that these permutations may differ considerably in their functionality in vivo. Individual HSs of the murine β -globin locus function additively to confer maximal expression of the β -like globin genes,²⁰ differing from the differential -1.8 kb and

–2.8 kb site activities at *Gata2*. Qualitatively distinct functions of GATA complexes were apparent at *Gata2*, highlighting the critical need to expand efforts to elucidate GATA motif function at a spectrum of endogenous loci.

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Authorship

Contribution: E.H.B. and S.H.O. conceived of the project; J.A.G., N.E.E. and J.W.S. built initial constructs, screened clones, and generated mice; J.W.S., T.F., K.D.J., and J.J.T. performed experiments; J.W.S. and J.J.T. analyzed data; and J.W.S., J.J.T., S.H.O., and E.H.B. wrote the manuscript.

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The current affiliation for J.W.S. is Biology Department, Williams College, Williamstown, MA.

Correspondence: Emery H. Bresnick, Department of Cell and Regenerative Biology, University of Wisconsin School of Medicine and Public Health, Wisconsin Institutes for Medical Research, Rm 4009, 1111 Highland Ave, Madison, WI 53705; e-mail: ehbresni@wisc.edu; and Jonathan W. Snow, Biology Department, Williams College, Williamstown, MA 01267, MA; e-mail: jonathan.snow@williams.edu.

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